

AMENDMENTS TO THE CLAIMS

The following is a complete listing of the claims submitted in this application, including the present status thereof and including any amendments made by this paper. Any claims canceled or withdrawn from consideration in this application have been canceled or withdrawn without prejudice or disclaimer of any subject matter therein, applicants specifically reserving the right to pursue any and all claims in continuing or divisional applications. By this paper, claims 187-188, 197-198 and 206-207 have been canceled; claims 183-186, 189-196, 199-205 and 208-211 have been amended. New claims 212-257 have been added.

Listing of Claims.

1-182(canceled).

183(currently amended). A non-human transgenic ~~vertebrate~~ mammal produced by the steps of:

- (a) administering by injection into a testis of a host male non-human ~~vertebrate~~ mammal a lentiviral vector comprising at least one xenogeneic polynucleotide encoding a gene product in operable linkage with a promoter, wherein said testis contains the germ cells of the male non-human ~~vertebrate~~ mammal, wherein said xenogeneic polynucleotide is xenogeneic to both said vector and said host, and wherein said germ cells are selected from the group consisting of spermatogonial

stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa; and

- (b) allowing the lentiviral vector comprising the xenogeneic polynucleotide encoding a gene product to be taken up by, and released into, the germ cells so that the released lentiviral vector and the polynucleotide are both incorporated into the genome of the germ cells of said male non-human ~~vertebrate~~ mammal.

184(currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 183, wherein the polynucleotide comprises at least one biologically functional gene.

185(currently amended). A progeny non-human transgenic ~~vertebrate~~ mammal, carrying in its germ cells a lentiviral vector comprising at least one xenogeneic polynucleotide sequence, said non-human ~~vertebrate~~ mammal being obtained by further breeding the male non-human ~~vertebrate~~ mammal of claim 183 with a female of the same species, and selecting the bred progeny non-human transgenic ~~vertebrate~~ mammal for the presence of the lentiviral vector comprising the xenogeneic polynucleotide in its genome.

186(currently amended). The progeny non-human transgenic ~~vertebrate~~ mammal of claim 185, being a male comprising native

germ cells carrying in their genomes at least one xenogeneic polynucleotide.

187-188 (canceled).

189 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 183, ~~which is a~~ wherein the mammal is selected from the group consisting of non-human primates, canines, felines, swine, farm and marine mammals, pachyderms, equines, murine, ovines and bovine ~~bovines, or a bird selected from the group consisting of ducks, geese, turkeys and chickens.~~

190 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 183, wherein the mammal is selected from the group consisting of wild and domesticated mammals.

191 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 183, wherein the mammal is a selected from the group consisting of farm ~~or~~ and marine ~~animal~~ animals.

192 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 183, wherein the mammal is selected from the group consisting of ~~a bull~~ bulls and ~~a pig, and the bird is a chicken~~ pigs.

193 (currently amended). A transgenic non-human ~~vertebrate~~ host mammal, comprising germ cells carrying in their genomes a lentiviral vector comprising at least one xenogeneic polynucleotide, said transgenic non-human ~~vertebrate~~ mammal having received an injection in its testis of male germ cells

comprising a lentiviral vector comprising at least one xenogeneic polynucleotide encoding a desired product and at least one polynucleotide encoding a genetic selection marker, wherein said xenogeneic polynucleotide is xenogeneic to both said vector and said host, said male germ cells comprising the polynucleotide being isolated or selected from a donor male non-human ~~vertebrate~~ mammal with the aid of the selection marker.

194 (currently amended). The ~~transgenic~~ non-human transgenic ~~vertebrate~~ mammal of claim 193 wherein the xenogeneic polynucleotide comprises at least one biologically functional gene.

195 (currently amended). A progeny non-human transgenic ~~vertebrate~~ mammal, carrying in its germ cells a lentiviral vector comprising at least one xenogeneic polynucleotide sequence, wherein said xenogeneic polynucleotide is xenogeneic to both said vector and said host, said non-human ~~vertebrate~~ mammal being obtained by further breeding the male non-human ~~vertebrate~~ mammal of claim 193 with a female of the same species, and selecting the bred progeny non-human transgenic ~~vertebrate~~ mammal for the presence of the lentiviral vector comprising the xenogeneic polynucleotide in its genome.

196 (currently amended). The progeny non-human transgenic ~~vertebrate~~ mammal of claim 195, being a male comprising native male germ cells transfected with a xenogeneic polynucleotide.

197-198 (canceled).

199 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 193, ~~which is a~~ wherein said mammal is selected from the group consisting of non-human primates, canines, felines, swine, pachyderms, equines, murine, ovines and ~~bovine,~~ ~~or a bird selected from the group consisting of ducks, geese,~~ ~~turkeys and chickens~~ bovines.

200 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 193, wherein the mammal is selected from the group consisting of wild and domesticated mammals.

201 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 193, wherein the mammal is a selected from the group consisting of farm ~~or~~ and marine ~~animal~~ animals.

202 (currently amended). The ~~vertebrate~~ non-human transgenic mammal of claim 193, wherein the mammal is selected from the group consisting of a ~~bull~~ bulls and a ~~pig~~ pigs, ~~and the bird is a chicken.~~

203 (currently amended). A non-human transgenic ~~vertebrate~~ host mammal, or its progeny, comprising a native germ cell carrying in its genome a lentiviral vector comprising at least one xenogeneic polynucleotide, wherein said xenogeneic polynucleotide is xenogeneic to both said vector and said host, said lentiviral vector comprising the polynucleotide having been incorporated into the genome of said germ cell through ~~the steps~~

of:

- (a) obtaining a male germ cell from a non-human vertebrate;
- (b) transfecting the germ cell in vitro with a lentiviral vector comprising at least one xenogeneic polynucleotide, wherein said xenogeneic polynucleotide is xenogeneic to both said vector and said host encoding a desired product, and optionally a polynucleotide encoding a genetic selection marker, at about or below the vertebrate's body temperature and for a transfection-effective period of time; and allowing the lentiviral vector and the xenogeneic polynucleotide encoding a desired product to be taken up by, and released into the germ cell.

204 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 203, wherein the xenogeneic polynucleotide comprises at least one biologically functional gene.

205 (currently amended). The progeny non-human transgenic ~~vertebrate~~ mammal of claim 203, being a male comprising native male germ cells transfected with a xenogeneic polynucleotide.

206-207 (canceled).

208 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 203, ~~which is a~~ wherein the mammal is selected from the group consisting of non-human primates, canines, felines, swine, pachyderms, equines, murine, ovines and ~~bovine~~.

~~or a bird selected from the group consisting of ducks, geese, turkeys and chickens~~ bovines.

209 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 203, wherein the mammal is selected from the group consisting of wild and domesticated mammals.

210 (currently amended). The non-human transgenic ~~vertebrate~~ mammal of claim 203, wherein the mammal is ~~a~~ selected from the group consisting of farm ~~or~~ and marine animal ~~animals~~.

211 (currently amended). The ~~vertebrate~~ mammal of claim 203, wherein the mammal is selected from the group consisting of ~~a bull and a pig~~ bulls and pigs, ~~and the bird is a chicken~~.

212 (new). A method of producing a transgenic non-human mammal progeny, the method comprising:

- (a) administering by injection into a testis of a male non-human mammal a transfection mixture comprising at least one polynucleotide encoding a gene product in operable linkage with a promoter, and at least one transfecting agent, other than a liposome/DNA complex, wherein said testis contains the germ cells of the male non-human mammal, and wherein said germ cells are selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary

spermatocytes, spermatids, and spermatozoa;

- (b) allowing the polynucleotide encoding a gene product to be taken up by, and released into, the germ cells so that the released polynucleotide is incorporated into the genome of the germ cells of said male non-human mammal thereby producing a transgenic non-human mammal; and
- (c) breeding said male transgenic non-human mammal with a female of the same species to produce a transgenic non-human mammal progeny.

213(new). A method of producing a transgenic non-human mammal progeny, the method comprising

- (a) administering by injection into a testis of a male non-human mammal a transfection mixture comprising a transferrin-polylysine enhanced adenoviral vector complexed with at least one polynucleotide encoding a gene product in operable linkage with a promoter, wherein said testis contains the germ cells of the male non-human mammal, and wherein said germ cells are selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene

spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;

- (b) allowing the adenoviral vector to be taken up by, and released into, the germ cells so that the polynucleotide is incorporated into the genome of the germ cells of said male non-human mammal thereby producing a transgenic non-human mammal; and
- (c) breeding said male transgenic non-human mammal with a female of the same species to produce a transgenic non-human mammal progeny.

214(new). A method of producing a transgenic non-human mammal progeny, the method comprising steps (a) and (b) of claim 212 wherein the transfection mixture further comprises at least one genetic selection marker, and the further steps of

isolating or selecting male germ cells whose genomes comprise at least one polynucleotide encoding a gene product and at least one polynucleotide encoding a genetic selection marker wherein the germ cells are from a donor male non-human mammal;

administering the isolated or selected germ cells to a testis of a recipient male non-human mammal; and

allowing the administered germ cells to lodge in a seminiferous tubule of the recipient male non-human mammal thereby producing a male non-human mammal useful for producing a transgenic non-human mammal;

breeding said recipient transgenic male non-human mammal with a female of the same species to produce a transgenic non-human mammal progeny.

215(new). A method of producing a transgenic non-human mammal progeny, the method comprising steps (a) and (b) of claim 213 wherein the transfection mixture further comprises at least one genetic selection marker, and the further steps of

isolating or selecting male germ cells whose genomes comprise at least one polynucleotide encoding a gene product and at least one polynucleotide encoding a genetic selection marker wherein the germ cells are from a donor male non-human mammal;

administering the isolated or selected germ cells to a testis of a recipient male non-human mammal; and

allowing the administered germ cells to lodge in a seminiferous tubule of the recipient male non-human mammal thereby producing a male non-human mammal useful for producing a transgenic non-human mammal;

breeding said recipient transgenic male non-human mammal with a female of the same species to produce a transgenic non-human mammal progeny.

216 (new). The method of claim 212, wherein the transfecting agent comprises a viral vector selected from the group consisting of a retroviral vector, an adenoviral vector, a mumps viral vector, a virus-derived DNA vector sequence, and a

mixture of the vectors thereof, wherein said transfecting agent facilitates uptake and release of the polynucleotide into the cytoplasm of the germ cells.

217 (new). The method of claim 216, wherein the viral vector is a transferrin-polylysine enhanced adenoviral vector or a lentiviral vector.

218 (new). The method of claim 217, wherein the lentiviral vector is a human immunodeficiency virus vector.

219 (new). The method of claim 212, wherein the viral vector is a Moloney murine leukemia virus-derived vector.

220 (new). The method of claim 212, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and wherein the gene product is expressed in the germ cells.

221 (new). The method according to claim 212, wherein the transfection mixture further comprises a male-germ-cell-targeting molecule consisting of c-kit ligand.

222 (new). The method of claim 212, wherein the promoter is selected from the group consisting of a c-kit promoter, a b-Myb promoter, a c-raf-1 promoter, an ATM (ataxia-telangiectasia) promoter, an RBM (ribosome binding motif) promoter, a DAZ (deleted in azoospermia) promoter, an XRCC-1 promoter, an HSP 90 (heat shock gene) promoter, and a FRM1 (from fragile X site) promoter.

223 (new). The method according to claim 212, wherein the transfection mixture further comprises an immunosuppressing agent.

224 (new). The method of claim 223, wherein the immunosuppressing agent is selected from the group consisting of cyclosporine and a corticosteroid.

225 (new). The method according to claim 212, wherein the injection is a percutaneous injection.

226 (new). The method according to claim 212, wherein the injection of the transfection mixture is into the vas efferens of the testis.

227 (new). The method according to claim 212, wherein the injection of the transfection mixture is into the seminiferous tubule of the testis.

228 (new). The method according to claim 212, wherein the injection of the transfection mixture is into the rete of the testis.

229 (new). The method according to claim 212, wherein the non-human mammal is selected from the group consisting of non-human primate, farm mammal, and marine mammal.

230 (new). A method of isolating or selecting a male germ cell transfected with at least one polynucleotide encoding a gene product and at least one genetic selection marker, comprising performing the method of claim 212, wherein the

transfection mixture further comprises at least one genetic selection marker; and

isolating or selecting a transfected male germ cell by detection of the genetic selection marker.

231 (new). The method of claim 213, wherein the transfecting agent comprises a viral vector selected from the group consisting of a retroviral vector, an adenoviral vector, a mumps viral vector, a virus-derived DNA vector sequence, and a mixture of the vectors thereof, wherein said transfecting agent facilitates uptake and release of the polynucleotide into the cytoplasm of the germ cells.

232 (new). The method of claim 231, wherein the viral vector is a transferrin-polylysine enhanced adenoviral vector or a lentiviral vector.

233 (new). The method of claim 232, wherein the lentiviral vector is a human immunodeficiency virus vector.

234 (new). The method of claim 213, wherein the viral vector is a Moloney murine leukemia virus-derived vector.

235 (new). The method of claim 213, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and wherein the gene product is expressed in the germ cells.

236 (new). The method according to claim 213, wherein the transfection mixture further comprises a male-germ-cell-targeting molecule consisting of c-kit ligand.

237 (new). The method of claim 213, wherein the promoter is selected from the group consisting of a c-kit promoter, a b-Myb promoter, a c-raf-1 promoter, an ATM (ataxia-telangiectasia) promoter, an RBM (ribosome binding motif) promoter, a DAZ (deleted in azoospermia) promoter, an XRCC-1 promoter, an HSP 90 (heat shock gene) promoter, and a FRM1 (from fragile X site) promoter.

238 (new). The method according to claim 213, wherein the transfection mixture further comprises an immunosuppressing agent.

239 (new). The method of claim 238, wherein the immunosuppressing agent is selected from the group consisting of cyclosporine and a corticosteroid.

240 (new). The method according to claim 213, wherein the injection is a percutaneous injection.

241 (new). The method according to claim 213, wherein the injection of the transfection mixture is into the vas efferens of the testis.

242 (new). The method according to claim 213, wherein the injection of the transfection mixture is into the seminiferous tubule of the testis.

243 (new). The method according to claim 213, wherein the injection of the transfection mixture is into the rete of the testis.

244 (new). The method according to claim 213, wherein the non-human mammal is selected from the group consisting of non-human primate, farm mammal, and marine mammal.

245 (new). A method of isolating or selecting a male germ cell transfected with at least one polynucleotide encoding a gene product and at least one genetic selection marker, comprising performing the method of claim 213, wherein the transfection mixture further comprises at least one genetic selection marker; and

isolating or selecting a transfected male germ cell by detection of the genetic selection marker.

246 (new). The method of claim 214, wherein the method further comprises co-administering Leydig or Sertoli cells to the testis with the isolated or selected germ cells.

247 (new). The method of claim 214, wherein the method further comprises isolating or selecting transfected Leydig or Sertoli cells, and co-administering to the testis with the isolated or selected germ cells.

248 (new). The method of claim 214, wherein the polynucleotide encoding the gene product is derived from the same species of non-human mammal as the recipient non-human mammal.

249 (new). The method of claim 214, wherein the polynucleotide encoding the gene product is derived from a human.

250 (new). A method of transferring autologous male germ cells and Leydig cells and/or Sertoli cells to the testis of a non-human mammal, comprising performing the method of claim 214, wherein the donor non-human mammal and autologous Leydig cells and/or Sertoli cells are co-administered with the male germ cells.

251 (new). The method of claim 214, wherein the donor non-human mammal is the same as the recipient non-human mammal.

252 (new). The method of claim 215, wherein the method further comprises co-administering Leydig or Sertoli cells to the testis with the isolated or selected germ cells.

253 (new). The method of claim 215, wherein the method further comprises isolating or selecting transfected Leydig or Sertoli cells, and co-administering to the testis with the isolated or selected germ cells.

254 (new). The method of claim 215, wherein the polynucleotide encoding the gene product is derived from the same species of non-human mammal as the recipient non-human mammal.

255 (new). The method of claim 215, wherein the polynucleotide encoding the gene product is derived from a human.

256 (new). A method of transferring autologous male germ cells and Leydig cells and/or Sertoli cells to the testis of a

non-human mammal, comprising performing the method of claim 215, wherein the donor non-human mammal and autologous Leydig cells and/or Sertoli cells are co-administered with the male germ cells.

257 (new). The method of claim 215, wherein the donor non-human mammal is the same as the recipient non-human mammal.